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Thin film micro arrays with immobilized DNA for hybridization analysis

F. Fixe[1,2], A. Faber[1], D. Gonçalves[1], D.M.F. Prazeres[1], R. Cabeça[2], V. Chu[2], G. Ferreira[3] and J.P. Conde[4]
[1]Center for Biological & Chemical Engineering, Instituto Superior Técnico, Lisbon, Portugal
[2] INESC Microsystems and Nanotechnologies, Lisbon, Portugal
[3] Faculdade de Engenharia de Recursos Naturais, Universidade do Algarve, Faro, Portugal
[4] Department of Materials Engineering, Instituto Superior Técnico, Lisbon, Portugal

ABSTRACT

In this work, a procedure to immobilize DNA probes on a microarray patterned on a flexible plastic substrate is developed. The method involves the chemical activation of a thin film surface, the introduction of amine functionality via a silanization step, the coupling of an adequate crosslinker and finally the immobilization of the DNA probe. The response of different thin-film materials and plastic substrates to the immobilization procedure is discussed. The DNA probes immobilized in the patterned pixels were then allowed to hybridize with complementary target DNA labeled with a fluorescent molecule. A prototype array of thin film pixels of SiO_2 functionalized by silanization deposited over a polyimide substrate is demonstrated.

INTRODUCTION

Biochips, particularly those based on DNA, are powerful devices that integrate the specificity and selectivity of biological molecules with electronic control and parallel processing of information. This combination will potentially increase the speed and reliability of biological analysis [1]. Thin-film microelectronic technology is especially suited for this purpose since it enables low-temperature processing and thus allows fabrication of electronic devices on a wide variety of substrates (glass, stainless steel, plastic, etc. [2]). Examples of current applications of DNA chips include genomic analysis to screen and identify single nucleotide polymorphisms (SNPs) or to sequence gene fragments, pathogen identification, and gene expression profiling [1].

The core of a DNA chip is a surface, usually flat, with DNA probe molecules spatially resolved and attached to it. This separation can be accomplished by using immobilization procedures that localize the probes to their exact site such as fine spotting [3], piezoelectric printing [4], electronic [5] and electrochemical addressing [6]. Alternatively, thin-film microelectronics technology can be used to deposit a material suitable for DNA attachment on an inert surface according to a given pattern. The use of passivation layers, although not described so often, can also be considered [7]. Other possible features of a DNA chip surface include the presence of microelectrodes beneath the DNA-containing areas. These can be used as sensing devices and to generate electric fields that promote the migration of oligonucleotides [5], hybridization and covalent binding [8]. Overall, the structure of a DNA chip is complex and its fabrication may include surface activation, passivation, patterning, and introduction of sensing devices and immobilization aids such as microelectrodes.

EXPERIMENTAL

Materials: Glass slides were obtained from Corning (7059). Polyimide (PI), polyethylene terephtalate (PET), polyetherether ketone (PEEK), polyethersulfone (PES), polycarbonate (PC)

and (PEN) plastic slides were obtained from Goodfellow. Oligonucleotides (both probes and targets) were purchased from Interactiva (Germany).

Silanization: The substrates and thin films tested were first cleaned with HNO_3, 5%(v/v), for 1 hr at 90ºC. In a later stage, a procedure using the less corrosive cholic acid (12 hr at room temperature) was developed to replace HNO_3, in order to minimize damage to thin film materials already present on the chip during the cleaning process. The surfaces were then silanized with 3-aminopropyltriethoxysilane (APTES) for 2 hr at 85ºC (figure 1).

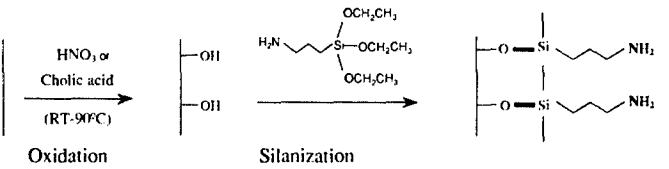

Figure 1. Introduction of amine functionality by surface modification with 3-aminopropyltriethoxysilane (APTES) after surface oxidation with HNO_3 or cholic acid.

Detection methods: The presence of NH_2 groups was evaluated by incubating the surfaces with 0.1 mg/ml fluorescein-5-isothiocyanate (FITC) for 30 min, followed by inspection with fluorescence microscopy. X-Ray Photoelectron Spectroscopy (XPS) and UV-VIS spectroscopy were also used to complement surface characterization.

Immobilization of DNA: After selecting the materials that could be silanized, DNA probes (17 bp) were immobilized using two chemical strategies. In the first strategy (figure 2a) surfaces were modified with the crosslinker glutaraldehyde. Next, DNA probes labeled with FITC and modified at the 5´-end with an amino group were linked to the terminal aldehyde group of the crosslinker (1 hr at room temperature). The second strategy (figure 2b) involved the modification of the activated films with the hetero-bifunctional cross-linker, sulpho succinimidyl 6-maleimidylhexanoate (sulpho-EMCS), for 2 hr at room temperature. Next, DNA probes labeled with FITC at the 3'-end and modified with a thiol group on the 5´-end were linked to the maleimide residues of the crosslinker. After immobilization for 3 hr the DNA probes were detected by fluorescence microscopy.

Hybridization: Non-labeled DNA probes were immobilized as described above using the cross-linker sulpho-EMCS. The surfaces were pre-hybridized with a solution containing Bovine Serum Albumin in order to avoid non-specific adsorption of the DNA target [9]. Hybridization was then carried out with a complementary (positive control) and a non-complementary (negative control) strand of DNA (target) labeled with FITC. The reaction was carried in 50 mM L-histidine at 37ºC overnight, followed by two washing steps with SSC (2x) + 0.1 % SDS. The detection was made by fluorescence microscopy.

Patterning: Arrays of SiO_2 on polyimide substrates were prepared in a clean room environment by direct write photolithography using a HeCd laser (λ= 440 nm) and reactive ion etching using a RF plasma of CF_4, O_2 and Ar.

Thin Film Deposition: The thin film deposition techniques used were plasma-enhanced chemical vapor deposition (silicon dioxide (SiO_2), amorphous silicon (a-Si:H), microcrystalline silicion (μc-Si:H), amorphous carbon (a-C:H), silicon nitride (SiN_x)) and magnetron sputtering (Al_2O_3, TiO_2, ZnO, SnO_2). All films were deposited at temperatures ≤ 150 ºC. A combination of

these layers allowed the definition of either functionalized islands on an inactive surface or windows of functionalized areas cut out of a passivation overlayer.

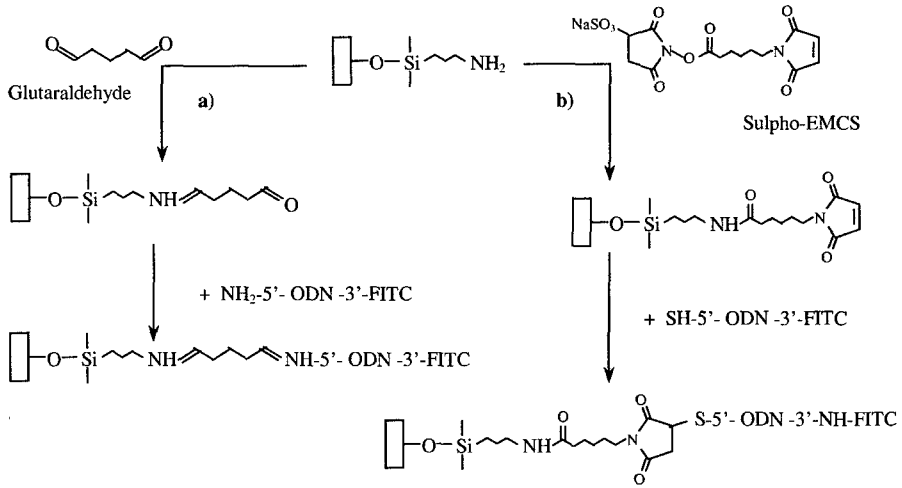

Figure 2 (a) and (b). Immobilization of FITC-labeled DNA probes onto silanized surfaces: modified with an amino group at the 5´-end were coupled via glutaraldehyde, and probes modified with a thiol group at the 5´-end were coupled via sulpho-EMCS, respectively.

RESULTS AND DISCUSSION

Activation (functionalization) and passivation layers: All substrates and thin films studied were subjected to the silanization procedure described in the previous section. The surfaces were then probed for NH_2 groups with FITC. The results obtained with different materials are described in table 1.

Table 1. Materials tested for activation by silanization (results obtained by fluorescence microscopy with FITC) (++: strong activation; +: activation; -: inert;?: auto-fluorescent substrate).

Substrate	Activation	Film*	Activation	Film*	Activation
Glass	++	SiO_2	++	SiNx	-
PI	-	TiO_2	++	Al_2O_3	-
PET	+	ZnO	++	C-Si p	-
PEEK	-	SnO	++	C-Si n	-
PES	-	a-Si:H	-	C-Si i	-
PC	?	a-C:H	-	Photoresist	-
PEN	-	μC-Si	-		

*The supporting substrate was either glass or PI

The results indicate that, apart from PET, all plastics tested are inert to the activation protocol used. These plastics are therefore appropriate substrates for the deposition of films of different materials. Polyimide was further selected for future work due to its good chemical and physical stability, as well as availability. A range of different thin film materials were deposited on top of PI and tested with the activation protocol. Only the oxide films were activated (i.e., functionalised by silanization), with the exception of Al_2O_3. These materials, and particularly SiO_2, were therefore selected as adequate surfaces for the attachment of DNA probes. On the other hand, Al_2O_3, silicon nitride and amorphous carbon (a-C:H) were chosen as passivation layers.

Most of the oxide films were also analysed by X-ray photoelectron spectroscopy (XPS), before and after silanization. As an example, figure 3 shows a XPS spectrum obtained for a silanized SiO_2 thin film deposited over PI. The binding energy for N (1s) (399,7 eV) is typical of NH_2 groups [10] and the binding energies of the others atoms C (1s), Si (2p) and O (1s) also confirm the functionalization of the silanized SiO_2 film surface, when compared with the XPS spectrum for non-silanized SiO_2.

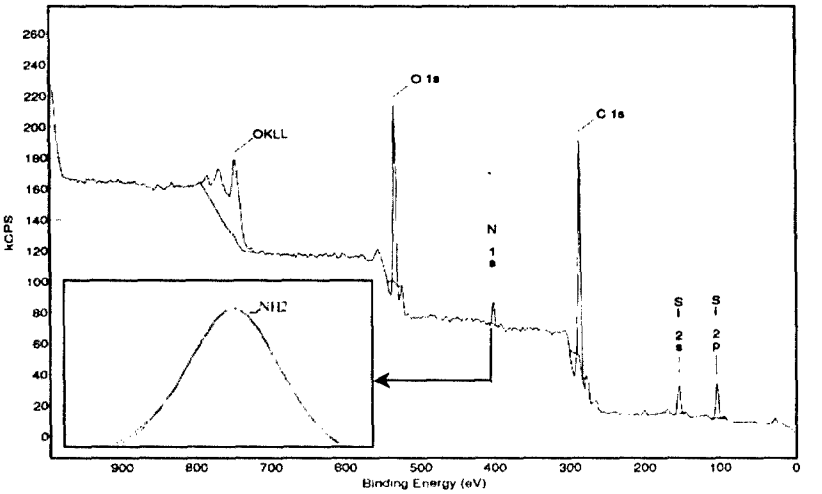

Figure 3. XPS scanning of a silanized SiO_2 thin film deposited over PI, showing the presence of nitrogen in the form of NH_2.

Patterning of thin film layers: After selecting the thin films that are functionalized by silanization and the substrates that are inert to this chemical procedure, two patterned surfaces were designed with islands of activated SiO_2 on PI (figure 4a) and active squares of glass delimited by a passivation layer of silicon nitride (figure 4b).

Immobilization of DNA probes: DNA probes were immobilized using two different cross-linkers, glutaraldehyde and sulpho-EMCS. In either case it was possible to attach the probes on non-patterned surfaces and on selectively activated areas defined by patterning (figure 5).

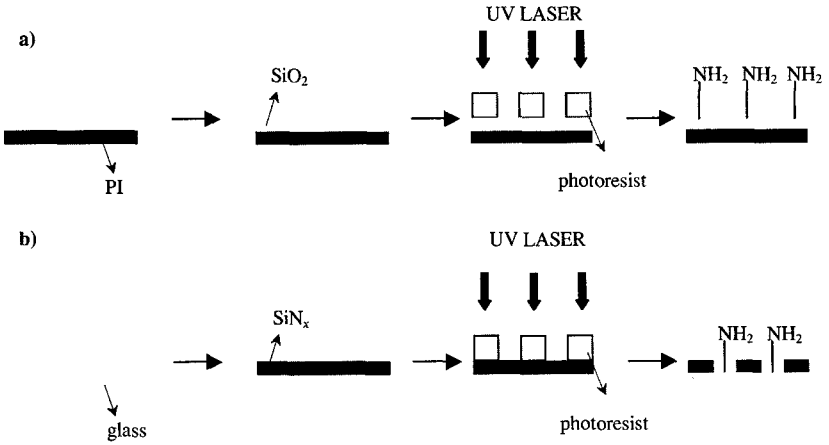

Figure 4 (a) and (b). Patterned surfaces, by photolithography, of active thin film layers (SiO_2) on the top of an inactive substrate (PI), or inactive layers (SiN_x) on an active (i.e., functionalized by silanization) substrate (Glass), respectively.

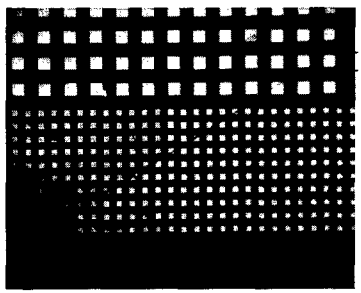

Figure 5. DNA probes immobilized on patterned active islands of SiO_2 deposited over PI. Sulpho-EMCS was used as a spacer arm and the probes were detected by fluorescence microscopy. The concentration of DNA was 1 μM and the immobilization time was 3 hr at room temperature.

Hybridization: Hybridization studies were made with DNA sequences complementary (positive control) and non-complementary (negative control) to the immobilized DNA probe. This was done in order to check if the immobilized probe can hybridize freely with a complementary strand, and also to make sure that the fluorescence signal obtained is due to hybridization alone and not to unspecific adsorption of the DNA target to the surface (Figure 6). The presence of fluorescence on the surface incubated with the complementary DNA target clearly indicates that hybridization has occurred. Furthermore, the absence of fluorescence in the surface that was incubated with the non-complementary DNA rules out the possibility of non-specific adsorption as a source of fluorescence.

Figure 6 (a) and (b). Hybridization of complementary and non-complementary DNA target, respectively, to DNA probes immobilized with sulpho-EMCS on a pattern of active glass exposed through windows in a passivating SiN_x passive layer

CONCLUSIONS

The work presents an enabling technology which applies low temperature thin film microelectronics processing to DNA chip and potentially other types of biological applications. Different materials were screened by a silanization chemistry for their ability to attach DNA probes. Glass and oxide films (SiO_2, TiO_2, ZnO, SnO_2) were modified, while plastic substrates such as polyimide (PI), thin films of silicon nitride, Al_2O_3, amorphous carbon and amorphous silicon were inert to the silanization procedure. On the basis of these results, chip prototypes were fabricated by photolithography, consisting of patterned arrays of functionalized thin film SiO_2 on a plastic substrate (PI) and islands of silanized glass passivated by a silicon nitride. DNA probes were then attached to the silanized areas using sulpho-EMCS as a hetero-bifunctional crosslinker. Hybridization of the DNA probes on the pixels with complementary DNA was demonstrated. This technology provides the basic building blocks for the integration of thin film electronic sensing and control with biological applications.

ACKNOWLEDGEMENTS

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